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Evaluation of the efficacy, acceptability and palatability of calcium montmorillonite clay used to reduce aflatoxin B1 dietary exposure in a crossover study in Kenya

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Abstract

Acute aflatoxin exposure can cause death and disease (aflatoxicosis) in humans. Aflatoxicosis fatality rates have been documented to be as high as 40% in Kenya. The inclusion in the diet of calcium silicate 100 (ACCS100), a calcium montmorillonite clay, may reduce aflatoxin bioavailability, thus potentially decreasing the risk of aflatoxicosis. We investigated the efficacy, acceptability and palatability of ACCS100 in a population in Kenya with recurring aflatoxicosis outbreaks. Healthy adult participants were enrolled in this double-blinded, crossover clinical trial in 2014. Following informed consent, participants ($n = 50$) were randomised to receive either ACCS100 (3 g day^{-1}) or placebo (3 g day^{-1}) for 7 days. Treatments were switched following a 5-day washout period. Urine samples were collected daily and assessed for urinary aflatoxin M1 (AFM_1). Blood samples were collected at the beginning and end of the trial and assessed for aflatoxin B1-lysine adducts from serum albumin (AFB_1-lys). AFM_1 concentrations in urine were significantly reduced while taking ACCS100 compared with calcium carbonate placebo ($\beta = 0.49$, 95% confidence limit = 0.32–0.75). The 20-day interval included both the placebo and ACCS100

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treatments as well as a washout period. There were no statistically significant differences in reported taste, aftertaste, appearance, colour or texture by treatment. There were no statistically significant differences in self-reported adverse events by treatment. Most participants would be willing to take ACCS100 (98%) and give it to their children (98%). ACCS100 was effective, acceptable and palatable. More work is needed to test ACCS100 among vulnerable populations and to determine if it remains effective at the levels of aflatoxin exposure that induce aflatoxicosis.

Keywords

Kenya; aflatoxin; aflatoxicosis; clay; ACCS100; uniform particle size Novasil (UPSN); Novasil; calcium montmorillonite

Introduction

Aflatoxins are harmful secondary metabolites of the moulds *Aspergillus flavus* and *A. parasiticus*, and they frequently contaminate agricultural produce such as cereals, oilseeds and root crops (Williams et al. 2004; Wild 2010). Acute exposure to aflatoxins can cause aflatoxicosis and liver failure (Wild & Gong 2010). Recurring aflatoxicosis outbreaks occur in Kenya's Eastern Province, where documented aflatoxicosis fatality rates approach 40% (Centers for Disease Control and Prevention (CDC) 2004). In Kenya, the first recorded aflatoxicosis outbreak occurred in 1981 (Ngindu et al. 1982). Between 2004 and 2014, subsequent outbreaks affected nearly 600 individuals and caused 211 deaths (personal communication with the Kenya Ministry of Health).

One possible approach to prevent illness associated with acute aflatoxin exposure is the use of a naturally occurring calcium montmorillonite clay. Air-classified calcium silicate 100 (ACCS100) is a refined calcium montmorillonite clay generally recognised as safe by the USFDA. The ACCS100 refining process is a simple fractionation method of the parent clay resulting in a product that contains the highest percentage of its particles within the size range of 45–100 μm, resulting in less quartz and large particles that might negatively influence palatability (Marroquin-Cardona et al. 2011). Importantly, ACCS100 clay can be included in the diet to adsorb aflatoxins tightly in the gastrointestinal tract, leading to decreased bioavailability.

High-affinity binding of aflatoxins to clay (Grant & Phillips 1998; Phillips et al. 2002), reduction of aflatoxin bioavailability, prevention of aflatoxicosis and safety at doses as high as 2% clay in the diet have been well-documented in animal studies (Phillips et al. 1988, 1995, 2002; Harvey et al. 1991, 1993; Mayura et al. 1998; Phillips 1999; Afriyie-Gyawu et al. 2005) and human clinical trials in the United States and Ghana (Wang 2008; Wang et al. 2005; Afriyie-Gyawu, Ankrah et al. 2008; Phillips et al. 2008; Mitchell et al. 2014; Pollock et al. 2016). Prior to clinical trials, a 2-week safety study was carried out in 50 US adults (Wang et al. 2005). This was followed by a 3-month Phase IIa clinical trial in adults, a 2-week crossover trial in adults and a 2-week safety study in children that found that the clay was safe for human consumption at levels as high as 0.25% in the diet and effective at reducing aflatoxin biomarkers in serum and urine. No significant differences in

haematology, liver and kidney function, electrolytes or minerals were found between placebo and active treatment groups. Furthermore, ACCS100 did not affect blood levels of micro- and macro-nutrients, such as vitamins A and E (Afriyie-Gyawu, Wang, et al. 2008).

In previous studies, clay was successfully delivered by capsule, or mixed in the diet. We envisaged that clay delivery in water might be more rapid and feasible during an outbreak period. Based on these studies, it was hypothesised that delivery of ACCS100 in water could be used to reduce biomarkers of aflatoxin exposure in urine and serum samples from participants living in high-risk regions of Kenya's Eastern Province. Our specific objectives in this study were to determine efficacy, acceptability and palatability of ACCS100 in water, using a crossover clinical trial in 50 health individuals.

Factors that can affect efficacy, acceptability and palatability include culture, ethnicity, diet, institutional policies, the amount of the contaminant and infrastructure. Because these factors can differ between countries and even within a single country, it is necessary to test an intervention in the specific at-risk population before implementation plans can be developed. Thus, the objectives were to assess these qualities of ACCS100 in Kenya's Eastern Province.

Materials and methods

Study design

The study protocol was approved by the Institutional Review Boards at the Kenya Medical Research Institute (KEMRI), Centers for Disease Control and Prevention (CDC), and Texas A&M University, bearing protocol IDs 2603, 6535.0 and 2013-0311F, respectively. The study's clinicaltrial.gov identifier is NCT02188953. The study started in July 2014 and ended in August 2014. A double-blinded, crossover clinical trial was conducted in which 50 participants were randomly assigned to group I or group II (Figure 1). Group I participants consumed 1 g of calcium carbonate placebo three times per day for 7 days. They did not consume any test material for 5 days (washout period), and completed the study with 1 g of ACCS100 three times per day for the last 7 days. Group II participants followed the same schedule, except they began with ACCS100 and finished with placebo. Dose ranged from 3 packet/40 kg to 3 packet/94.5 kg (Table 1). The dose in the current study has been shown to be safe and effective in several human trials. Since the ultimate goal of this mitigation strategy is for emergency use during outbreak situations, a single minimal effective dose in adults and children, respectively, would be the most field-practical.

Intervention

Texas EnteroSorbents, Inc. provided ACCS100 and placebo as 1-g foil sachets packaged for individual use. Participants were instructed to consume one sachet with each of their three main meals by diluting the sachet in water provided by the study; thus, participants would consume 3 g of ACCS100 or placebo per day during the study arms. The sachets were coloured pink or green to designate the content (ACCS100 or placebo respectively). The study investigators and participants were both blinded regarding treatment type. Each day

during the study each participant was provided a sterile urine cup, three treatment sachets and three 500-ml bottles of clean water.

Study population

The study was implemented in Kalimani and Kamboo villages of Kamboo sub-location in Makueni county, Eastern Kenya. The region is predominately agricultural and composed of subsistence farming, and residents eat primarily a maize-based diet. These villages were chosen because they had experienced multiple aflatoxicosis outbreaks in the prior decade.

Sampling and enrolment

Field teams employed convenience sampling and went door to door to recruit participants, enrolling one adult per household. Inclusion criteria consisted of the following: (1) age ≥ 18 years; and (2) consumed maize and/or groundnuts at least four times per week. Once a participant was identified, written informed consent was obtained. Consent forms were supplied in the local language (Akamba) as well as the national language (Kiswahili). Participants could decline to participate in any part of the study and were free to withdraw at any point. Upon completion of the study, participants were reimbursed with cooking items worth approximately 400 Kenya shillings (US$4).

All study participants were provided with detailed study objectives and procedures. After consent was provided, each person's medical history, height and weight were recorded and their current health status was assessed through a physical example and onsite urine testing. Urine specimens were analysed for protein and glucose levels using Chemistrip®2 GP test strips from Roche Diagnostics (Indianapolis, IN, USA). These tests helped to rule out many health conditions involving the metabolic system, e.g., diseases of the liver (e.g., hepatitis, cirrhosis, etc.), kidney (e.g., nephrotic syndrome, renal failure, etc.) and thyroid gland (e.g., hypothyroidism, etc.). Participants who reported a history of thyroid disease, heart disease, lung disease, kidney disease, gastrointestinal disease or diabetes, or who had protein or glucose levels outside the normal range were informed of their result and excluded from the study. Female participants aged 18–49 were assessed for pregnancy status onsite using Sure-Vue® urine human chorionic gonadotropin strips (Fisher Healthcare, Pittsburgh, PA, USA). Participants who were pregnant were excluded because of unknown effects of ACCS100 on human pregnancy (although studies on pregnant rats have reported no serious adverse effects). Participants not excluded were enrolled in the study and randomly assigned to group I or II.

Data collection

An enrolment survey was administered that included questions regarding the duration of residency in the village, source of maize and the shelf-life of their maize stock.

Participants provided a first morning void urine sample at baseline, and again for each of the 7 days of each treatment arm. Given the short half-life of aflatoxin in the urine (1–2 days) and the transit time of ACCS100 in the gastrointestinal tract (1–3 days), aflatoxin M1 should have completely rebounded during the wash-out period. Urine samples were collected,

aliquoted and frozen each morning, then kept frozen until laboratory analysis. Two blood samples were also collected from each participant at baseline (day 0) and completion (day 20) of the trial for AFB_1-lys analysis.

Following each treatment arm, a questionnaire was administered that asked participants to rate the taste, aftertaste, smell, texture, appearance and colour of the sachet contents they had been consuming using a five-point Likert type scale (1 = really bad; 5 = really good).

Study monitors completed a worksheet each day during the study to record participant adherence to protocol (i.e., daily use of ACCS100 or placebo), the occurrence of side effects and diet. For adherence, daily use of ACCS100 or placebo was obtained by asking participants to self-report their consumption, and by collecting empty treatment sachets. For side effects, additional information was collected regarding side-effect severity (i.e., mild, moderate or severe), time of day the side effect occurred (i.e., a.m., noon or p.m.), and whether or not the participant sought treatment.

Acceptance was also assessed by administering a questionnaire at the end of the study to collect the participant's perceptions of ACCS100 and whether they would be willing to consume ACCS100 in future, or whether they would be willing to have their children consume ACCS100.

Determination of urinary AFM_1

The exposure of AFM_1 in humans is by ingestion of food contaminated with AFB_1 which is then metabolised to AFM_1, or consumption of contaminated milk and dairy products. Urine samples were analysed at Texas A&M University. Analysis of urinary AFM_1 levels followed methods reported by Groopman et al. (1992) with the modifications of Sarr et al. (1995) and Wang et al. (1999). Urine samples were centrifuged at 2300 rpm, and 5.0 ml of supernatant were collected, acidified with 0.5 ml of 1.0 M ammonium formate (pH 4.5) and diluted with water to a total volume of 10.0 ml. Samples were then loaded onto a 3-ml preparative Aflatest® WB immunoaffinity column (VICAM, Watertown, MA, USA) at a flow rate of 1 ml min^{-1}. Following washing of the column, the aflatoxin fraction was eluted from the column with 2 ml of 80% methanol, dried under N_2 and resuspended in 200 μl of a 1:1 solution of methanol:20 mM ammonium formate. Samples were analysed using a Waters HPLC system (Waters Corporation, Milford, MA, USA) with fluorescence detection capabilities. A 250 × 4.6 mm Luna C-18 column with pore size 100 Å and particle size 5 μm (Phenomenex, Torrance, CA, USA) was used to resolve AF metabolites. The mobile phase consisted of 22% ethanol buffered with 20 mM ammonium formate (pH 3.0) in water. Isocratic elution of the mobile phase for 20 min at a rate of 1 ml min^{-1} allowed for proper chromatographic separation. External AFM_1 standards were prepared weekly and injected following every five injections of samples. The LOD for this method was 12 pg ml^{-1} of urine for AFM_1. Random samples were aliquoted for additional verification using a Waters Acquity H-Class UPLC-MS/MS. Separation was achieved using a 2.1 × 50 mm Acquity UPLC BEH C18 column with a particle size of 1.7 μm. Isocratic separation was achieved with 70% water buffered with 1% formic acid and 30% ACN buffered with 1% formic acid. Samples (10 μl) were injected onto the column and the elution rate was 0.325 ml min^{-1}. The

column effluent was directly coupled to the MS, which was operated in the positive electrospray ionisation mode. MS/MS conditions were optimised for AFM_1 and based on Warth et al. (2012). The precursor ion was set to 329.00 Da and the two product ions were 273.00 Da (quantifying ion) and 259.1 Da (qualifying ion). Urinary AFM_1 concentrations were expressed as pg mg^{-1} creatinine to correct for variations in urine dilution among samples. Creatinine concentrations were measured at Baylor Scott & White Hospital (Temple, TX, USA).

Determination of serum AFB_1-lysine adduct level

Previous measurements of aflatoxin exposure in Kenya have been based on the serum aflatoxin B_1-lysine adduct from serum albumin (AFB_1-lys), a biomarker for long-term aflatoxin exposure. This allowed us to compare aflatoxin exposure with levels seen during previous aflatoxicosis outbreaks. The CDC's National Center for Environmental Health Division of Laboratory Sciences analysed serum specimens for AFB_1-lys adduct, which consisted of two measurements: (1) analysis AFB_1-lys by LC-MS/MS (McCoy et al. 2005); and (2) albumin measurement. To allow the release of AFB_1-lys from albumin, protein in serum specimens was digested in the presence of stable-iso-topically labelled internal standard ($^2H_4$-AFB_1-lys) for at least 15 h at 37°C by use of a commercially available mixture of proteinases (Pronase™). AFB_1-lys and $^2H_4$-AFB_1-lys were then extracted by use of mixed-mode anion exchange reversed-phase SPE. Each SPE eluate was evaporated, reconstituted in mobile phase and injected onto a reversed-phase C_{18} column. AFB_1-lys was chromatographically separated from other compounds using gradient mobile phase. Both AFB_1-lys and $^2H_4$-AFB_1-lys were detected with positive electrospray ionisation (ESI) in SRM mode using tandem quadrupole mass spectrometry (McCoy et al. 2005). Quantitation was based on peak area ratios interpolated against a seven-point aqueous linear calibration curve with 1/x weighting. The calibration range for serum AFB_1-lys was 0.025–10 ng ml^{-1}. There are no established critical call values for serum AFB_1-lys concentrations, i.e., there are no defined concentration thresholds distinguishing a normal or acceptable serum AFB_1-lys concentration from one that would be considered abnormal or life threatening. The LOD for AFB_1-lys was 0.02 ng ml^{-1}. Serum albumin was analysed on the Hitachi Modular P clinical analyser using the Roche® colorimetric assay. The LOD for albumin was 0.2 g dl^{-1}. Human serum albumin – and subsequently albumin-corrected serum AFB_1-lys – has a half-life of approximately 20 days. Thus, detection of AFB_1-lys in this assay suggests a likelihood of exposure to aflatoxin within the previous 1–2 months.

Statistical methods

We used Epi Info™ 7 (CDC, Atlanta, GA, USA) for data entry and SAS Enterprise Guide version 4.3 (SAS Institute, Cary, NC, USA) for data analysis. We performed all statistical analyses blinded, and we considered $p < 0.05$ to be statistically significant.

We compared demographic variables by group using paired *t*- and chi-square tests. We compared the palatability by treatment using Wilcoxon rank-sum test, and compared serum AFB_1-lys levels between days 0 and 20 using the Wilcoxon signed-rank test. We assessed intra-individual correlation in urinary AFM_1 levels using a Spearman correlation coefficient.

To assess efficacy, we first created an outcome variable for each participant. The outcome variable was the average of a participant's urinary AFM_1 level during the 7 days of follow-up (i.e., days 2–8) for each treatment. Urinary aflatoxin levels below the LOD were substituted with the LOD divided by the square root of 2 before being averaged. These averages were then log-transformed. Thus, each participant had two summary outcome variables: log-transformed average urinary AFM_1 during treatment; and log-transformed average urinary AFM_1 during placebo. We then fitted a general linear model with fixed effects for subject, treatment and period. We performed a modified intent-to-treat analysis, meaning that we performed an intent-to-treat analysis on all individuals who completed the study.

Results

Study population and demographics

In order to enrol the study population of 50 participants, a total of 68 potential participants were consented and assessed. Eighteen participants were not enrolled because they did not meet the inclusion criteria (Figure 1). Study retention for randomised participants was 98%. One (2%) male participant dropped out on day 3 after being randomised and completing two treatment sessions. Thus, we included 49 participants in the statistical analyses.

The majority of participants were female (n = 36, 72%) (Table 1). Participants ranged in age from 21 to 75 years (mean = 39 years). Participant sex, age, weight, height and amount of time living in the village did not differ by group. Participants consumed an average of 1.9 maize-containing meals per day; this did not differ by treatment (placebo, 1.8 maize-containing meals per day; ACCS100, 1.9 maize-containing meals per day).

Compliance

The majority of participants consumed all 21 sachets during both the ACCS100 (n = 45) and placebo (n = 44) treatment. The majority (n = 46) of participants always ingested the sachet with water; four individuals reported consuming a sachet without water (but with food) once during the study. There were 23 participants who consumed a sachet without food (but with water) between one and three times during the study.

Efficacy

Forty-nine participants contributed data to both arms of the study and thus were included in the efficacy analyses. Participants provided 784 (98%) of the potential 800 urine samples. Overall, 48% of samples contained detectable levels of urinary AFM_1 (range = < LOD–1986 pg mg^{-1} creatinine).

Baseline urinary AFM_1 levels also did not vary statistically by treatment. There was no statistical correlation in urinary AFM_1 aflatoxin levels when comparing baseline of arm 1 with the baseline of arm 2, or when comparing day 1 of arm 1 with day 2 of arm 1.

Table 2 depicts geometric mean average urinary AFM_1 levels during follow-up (days 2–8)by arm and group. Our general linear model found that geometric mean urinary AFM_1 was lower during ACCS100 compared with placebo (β = –0.7093, 95% confidence limit = –1.14

to –0.28; $p < 0.01$), while controlling for subject and period. Once exponentiated to account for the log transformation, the results indicate that urinary AFM_1 was approximately half during treatment compared with placebo (0.49, 95% confidence limit = 0.32–0.75).

Figure 2 illustrates daily urinary AFM_1 levels by treatment. As indicated by the error bars, there was not a statistically significant difference between ACCS100 and placebo when looking at any individual day.

The average level of consumption of calcium montmorillonite clay was 3 g day^{-1}. Previous research has demonstrated that 3 g day^{-1} of clay was the minimal effective dose that significantly decreased the AFM_1 bio-marker (Wang et al. 2008). This dose of clay (3 g day^{-1}) is equivalent to 0.25% w/w in the adult Ghanaian diet, based on an intake of approximately 1200 g day^{-1}.

Analysis of serum AFB_1-lys levels

Thirty-nine participants provided serum data at both time points and thus were included in this analysis (Figure 3). Serum aflatoxin median levels exhibited a statistically significant decrease from day 0 ($n = 39$; median = 9.3 pg mg^{-1} albumin) to day 20 ($n = 39$; median = 6.4 pg mg^{-1} albumin; $p < 0.01$). The day 0–20 interval includes both the placebo and ACCS100 treatments, as well as the wash out.

Palatability

There was a statistically significant difference in smell by treatment, with participants on average rating the placebo as 0.25 Likert points better than ACCS100 ($p < 0.05$). While the placebo and treatment had slight difference in colour, there were no statistically significant differences in ratings of taste, aftertaste, appearance, colour or texture by treatment (Table 3).

The majority of participants rated aftertaste (98%), texture (97%), appearance (99%), smell (99%), taste (99%) and colour (99%) as either okay, good or really good for both the placebo and ACCS100. No one reported appearance, colour and taste as bad for the placebo. However, ACCS100 received a ‘bad’ rating from one participant (2.2%) for appearance, one (2.2%) for colour, one (2.2%) for taste and one (2.2%) for texture. Two (4.2%) people rated the placebo texture as bad.

Acceptability

Forty-seven participants completed the end-of-study questionnaire. Most participants (96%) had heard of aflatoxin prior to the study, and most (91%) worried about becoming sick as a result of exposure to afla-toxin. Two-thirds of participants (67%) knew of someone who had become sick from aflatoxin exposure in the past, and 9% believed they themselves had become sick from aflatoxin exposure at some point.

Most participants (98%) did not have any concerns about ACCS100 and would be willing to take ACCS100 (98%) or give it to their children (98%) if they knew it would protect them from aflatoxicosis. The majority of participants (72%) reported that they would prefer to

take the clay in water as they had done during the study; the other commonly mentioned option was taking the clay plain (i.e., licking it; 13%). Participants would be willing to take the clay for at least 2 weeks (40%) or as long as recommended (38%). Similarly, participants would be willing to let their children take the clay for at least 2 weeks (36%) or as long as recommended (47%).

Adverse events

Approximately one-quarter of participants reported at least one adverse event when taking placebo (n = 12, 26%) or ACCS100 (n = 14, 28%). The most commonly reported adverse events reported while on placebo were nausea (14%) and abdominal discomfort (8%); for ACCS100, they were abdominal discomfort (14%) and increased appetite (8%) (Table 4). Other side effects reported at least once were diarrhoea (reported once, while on ACCS100), and dizziness with headache (reported once, while on ACCS100). These percentages were calculated by dividing the number of times an adverse event was reported by the total potential number of times when it could have been reported. There were no statistically significant differences in the reporting of adverse events by treatment.

No adverse events were graded as severe. Approximately one-third of side effects on ACCS100 (n = 6, 35%) and placebo (n = 7, 39%) were rated as moderate.

Discussion

We found no relationship between self-reported adverse events and treatment type. Two of the most commonly reported side effects in our study were abdominal discomfort and nausea, and both have been reported in previous studies. One difference in the present study is that a small number of participants reported increased appetite. It is possible participants may have paid more attention to their level of hunger because we performed our study during a time of relatively high food insecurity.

Another difference between our study and earlier work is the delivery mechanism. Previously, ACCS100 had been delivered in food, or as a pill. This is the first study to show that clay can be palatable and acceptable when mixed with water. This is significant because clay delivery in water might be more rapid and feasible during an outbreak period compared with delivery in food or capsules.

We found ACCS100 to be effective in reducing aflatoxin bioavailability. Participants in both groups had lower urinary AFM_1 levels while taking ACCS100 compared with placebo. During a similar crossover study in Ghana, participants also exhibited lower urinary AFM_1 levels while taking clay compared with placebo, though the effect was statistically significant in only one group (Mitchell et al. 2013). Baseline urinary AFM_1 levels were approximately 80 times higher during the crossover trial in Ghana than what we found in Kenya, suggesting that participants in our study had lower exposure to aflatoxin. Importantly, the dose of ACCS100 (i.e., 0.25% w/w) represents a minimal effective concentration based on extensive earlier work in animals and has been kept at this level as a safety measure during human trials. Studies have shown that the clay is tolerable in animals

as high as 2.0% w/w. It is possible that a higher dose of clay will be more efficacious during an outbreak or emergency. Further work is warranted to investigate the dosimetry of ACCS100.

Median AFB_1-lys levels in our study participants (9.3 pg mg^{-1} albumin on day 0 and 6.4 pg mg^{-1} albumin on day 20) were similar to median levels reported during a previous study that measured AFB_1-lys levels in Kenya's Eastern Province during a non-outbreak period (7.9 pg mg^{-1} albumin) (Yard et al. 2013). As expected, AFB_1-lys levels were much lower during this study compared with levels reported among patients with presumed acute aflatoxin toxicity during aflatoxicosis outbreaks in Kenya in 2004, 2005 and 2010, when geometric mean levels ranged from 120 to 1200 pg mg^{-1} albumin (Azziz-Baumgartner et al. 2005).

The decrease in serum aflatoxin from 9.3 pg mg^{-1} albumin on day 0 and 6.4 pg mg^{-1} albumin on day 20 may be the result of treatment with ACCS100 or might reflect a decrease in dietary exposure to aflatoxin. Longer-term studies using this biomarker are warranted.

There are multiple strategies to prevent aflatoxin contamination and exposure. A variety of long-term solutions include reducing aflatoxin contamination in maize through improved harvesting, drying and storage (Turner et al. 2005); planting resistant cultivars (Hell et al. 2008), bio-control (Cotty et al. 2007; Yin et al. 2008), and/or a gradual shift to a more diverse diet (Wu et al. 2014). However, even with the implementation of these strategies, it is still difficult to eliminate aflatoxins completely. Thus, short-term interventions are needed in Kenya to respond to afla-toxin outbreaks.

A number of further research questions need to be answered before ACCS100 could be incorporated into an outbreak-response strategy. First, we do not know if ACCS100 will be efficacious in the higher exposure levels like those seen during outbreaks. Second, more work is needed to test the safety, efficacy, acceptability and palatability among vulnerable populations, such as children, individuals in poor health and pregnant women, who are often most at risk for aflatoxicosis. Third, though we have seen evidence of ACCS100 reducing aflatoxin bioavailability, we do not know if that will correlate to improved health outcomes. Fourth, we do not know if the reduction in bioavail-ability is fast enough to prevent toxicity.

ACCS100 has the potential to be incorporated in outbreak-response activities to prevent poisoning and protect people during high-risk periods. This pilot study was designed to confirm the efficacy, acceptability and palatability of ACCS100 in a high-risk population in Kenya.

It had a few limitations, though. Compliance with the study protocol was measured via self-reported data, which may have biased results towards the null. However, this appeared not to have been an issue, as we still observed a statistically significant decrease of AFM_1 levels when participants were on ACCS100 compared with placebo. There was no untreated or placebo-only group, so we do not know if the results were due to a trend in exposure or clearance, or if the treatment was effective.

This study was conducted in a region of Kenya that had a very high awareness of aflatoxin due to prior outbreaks. It is possible that other regions of Kenya may not have been as accepting of ACCS100 or found it as palatable. However, this is the region where ACCS100 could be used in future due to its history of high exposure and previous outbreaks.

Although many questions remain, the results from this pilot study are encouraging. They suggest that ACCS100 would be acceptable in Kenya's Eastern Province. The decrease in urinary AFM_1 biomarkers during ACCS100 treatment should be further explored to determine if ACCS100 could be a potential treatment to protect individuals at risk for aflatoxin poisoning.

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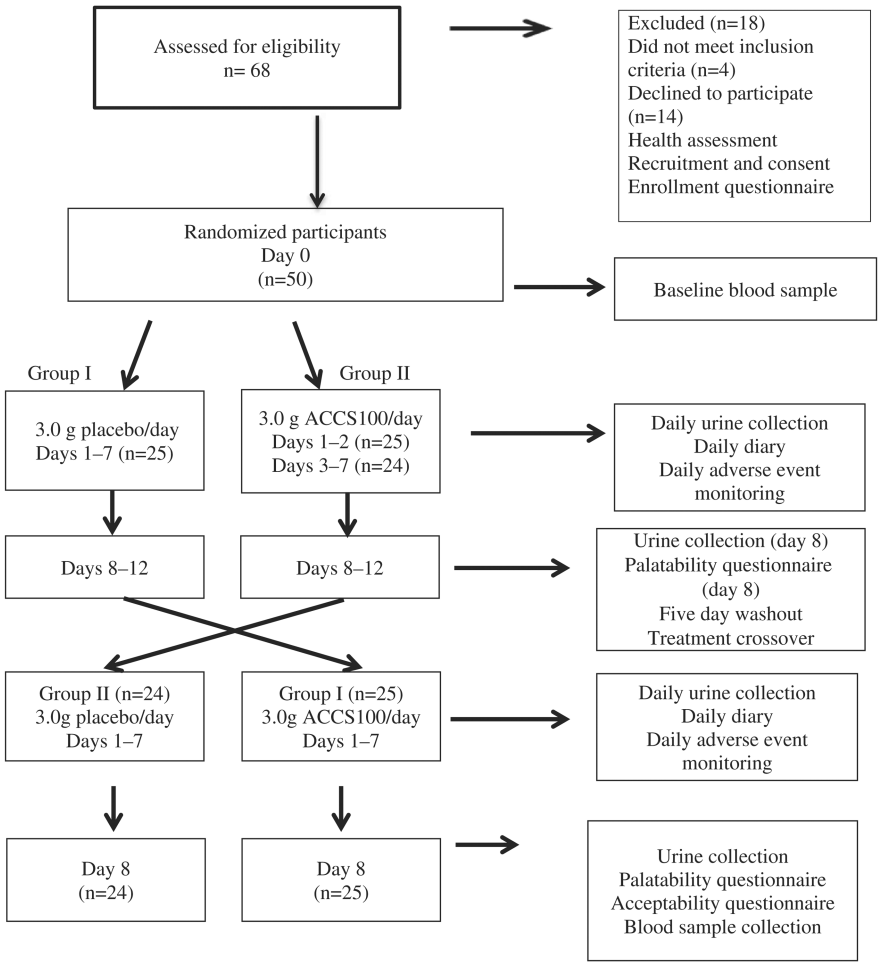

Figure 1.
Overall design and participants flow for the ACCS100 crossover study.

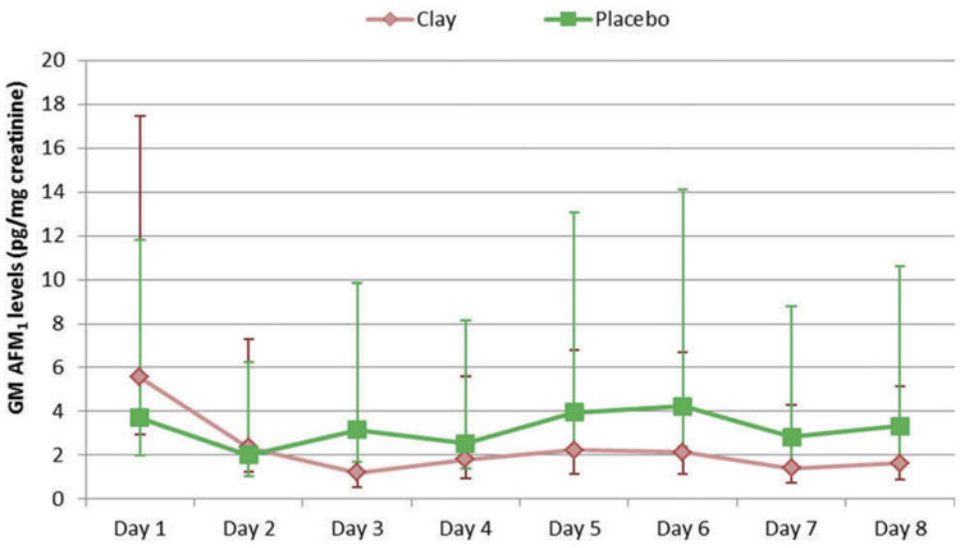

Figure 2.

(colour online) Geometric mean and 95% confidence intervals of urinary aflatoxin AFM_1 (pg mg^{-1} creatinine) by day.

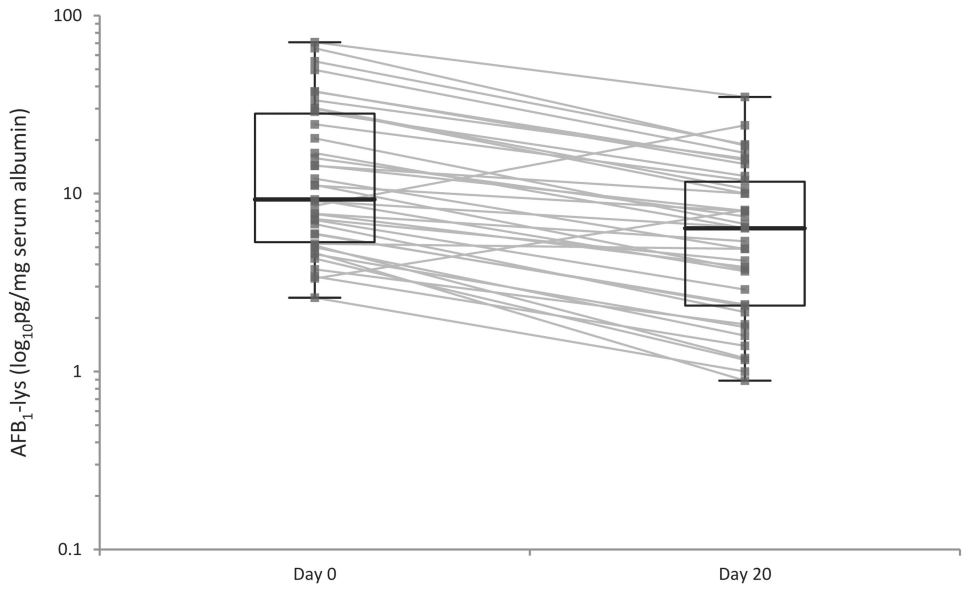

Figure 3.

Box whiskers/connected dot plot of AFB_1-lys for 39 participants on days 0 and 20 of follow-up. The day 0–20 interval includes both the placebo and ACCS100 treatments, as well as the wash out.

Table 1

Study population demographics, by group.

Demographic	Overall	Group I (placebo then ACCS100)	Group II (ACCS100 then placebo)
Sex (%)			
n	50	25	25
Female	36 (72%)	17 (68%)	19 (76%)
Male	14 (28%)	8 (32%)	6 (24%)
Age (in years)			
n	48	25	23
Range	21–75	21–68	21–75
Mean	39.3	40.6	37.9
SD	12.3	12.8	11.9
Weight (kg)			
n	47	23	24
Range	40.0–94.5	41.5–88.0	40.0–94.5
Mean	58.4	61.8	55.1
SD	7.8	12.7	10.8
Height (cm)			
n	47	23	24
Range	142.2– 185.4	151.0–185.4	142.2–173.5
Mean	161.6	163.0	160.2
SD	7.8	8.1	7.5
Duration of stay in the village (years)			
n	50	25	25
Range	1–55	4.0–55.0	1.0–51.0
Mean	20.0	22.5	17.4
SD	15.2	16.0	14.3

Table 2

Urinary aflatoxin AFM_1 concentrations (pg mg^{-1} creatinine) during follow-up (days 2–8), by group and arm.

	Arm 1	**Arm 2**
Group I		
n	174	173
Treatment	Placebo	Treatment
AFM_1:GM (95% CI)	16 (8.2–31)	7.4 (4.2–13)
Group II		
n	170	168
Treatment	Treatment	Placebo
AFM_1:GM (95% CI)	10 (4.0–26)	18 (7.2–44)

Note: The general linear model compared log average AFM_1 levels during days 2–8 between ACCS100 and placebo: β (95% confidence limit) = –1.14 to –0.28; $p < 0.01$.

Table 3

Palatabilitya ratings reported by treatment.

	Placebo, *n* = 48	**ACCS100, *n* = 45**
Aftertaste	3.7	3.8
Appearance	3.7	3.7
Colour	3.8	3.9
Smell[b]	4.0	3.8
Taste	4.0	4.1
Texture	3.8	3.6

Notes:

[a]Ratings reflect a five-point Likert scale (1 = really bad, 2 = bad, 3 = okay, 4 = good, 5 = really good).

[b]$p < 0.05$ comparing ACCS100 with placebo using a Wilcoxon rank-sum test.

Table 4

Adverse events reported, by treatment.

Side effect	Placebo (participants: n = 50) (possible times: n = 350)[a]	ACCS100 (participants: n = 49) (possible times: n = 343)
Abdominal discomfort		
Number of participants reporting	4 (8%)	7 (14%)
Number of times reported	5 (1%)	9 (3%)
Nausea		
Number of participants reporting	7 (14%)	2 (4%)
Number of times reported	8 (2%)	4 (1%)
Increased appetite		
Number of participants reporting	3 (6%)	4 (8%)
Number of times reported	6 (2%)	4 (1%)

Note:

[a]Possible times was calculated by multiplying the number of participants by the number of days of treatment.